IJBST

ISSN: 0974 – 3987
IJBST (2011), 4(11):77-81

# Quantitative and Qualitative Comparison of DNA Extracted from Seeds & Leaves of *Ocimum* Species

Subarna Roy*, Archana Saini, Dushyant Kumar, Sagnik Chakraborty, Harsha Hegde, Sanjiva D Kholkute

Regional Medical Research Centre, Indian Council of Medical Research, Belgaum, Karnataka 590010, India.
roys@icmr.org.in

**ABSTRACT:**
Leaves are the tissues of choice for isolation of genomic DNA from plants. However, fresh leaves are required for the purpose and require maintenance of cold chain during transportation. Seeds are generally hardy, have long storage life and do not require transportation in cold chain after collection. To evaluate the suitability of seeds as alternate source of DNA, the quality and quantity of DNA extracted from leaves and seeds of three species of the widely used medicinal plant genus *Ocimum* were compared. The commonly used CTAB/NaCl method, modified from Keb-llanes method, was used for isolation of DNA from both tissues of the *Ocimum sanctum* L., *O. basilicum* L., and *O. gratissimum* L. The yield of DNA from seeds of each species was found to be significantly higher than the corresponding masses of leaves from the same plant. The quality of DNA isolated from both seeds and leaves was found equally suitable for PCR-based downstream applications like RAPD, and ISSR fingerprinting. Seeds of medicinal herbs and shrubs, like *Ocimum*, if available, can therefore serve as better specimens for DNA extraction.

**Key words:** plant DNA, extraction, tissue, leaf, seed

## INTRODUCTION

Medicinal plants, like other commercial crops, have wide genetic variability and are being exploited from time immemorial. Plant genetic variability analysis is very useful to breed and develop improved high yielding varieties and also for their commercial utilization. Thus, medicinal plants have also attracted the attention of molecular biologists at large [1-4]. DNA based methods are increasingly being used in identification and authentication of medicinal plants as these are believed to be more reliable and consistent [5,6]. DNA fingerprinting and barcoding is done for differentiation of plants at generic, specific and even at the variety levels [1,2,7,8]. It is also used in studies in population structure and population genetics as well as in studies on evolution [9-11]. For most of these studies, high quality of genomic DNA or total DNA is a prerequisite. Although several kit-based methods are now available in the market, isolation of genomic DNA by the conventional CTAB/NaCl method still remain the most widely used method of isolation of good quality DNA for subsequent downstream applications like DNA fingerprinting in most laboratories. In plant molecular biology, leaves are considered the tissue of choice for extraction of genomic DNA for these downstream applications. For extraction of DNA, the leaves need to be either freshly plucked or fresh leaves transported from sampling sites to the concerned laboratories maintaining cold chain and storage, if required, in suitable freezers. This often becomes practically difficult particularly when the sampling site is far away from the laboratory, as is the case for most wild/forest plants. Often important samples are spoiled due to lack of proper transportation and storage facilities. Also the yield of genomic DNA obtained from leaves turn unsatisfactory in the event of insufficient quantity of leaves or spoilage of leaves in transit. Seeds, on the contrary, can be easily stored for long durations without any specific preserving conditions and therefore, if available, has the potential to serve as excellent source of plant DNA. Thus the present study was undertaken to evaluate the utility of seeds as alternate sources of DNA with the genus *Ocimum* (family Lamiacea) which include economically very important group of herbaceous plants, with well established therapeutic potentialities [12-18].

## MATERIALS AND METHODS

**Plant materials**:
A few fresh leaves and seeds from five different individuals of each of three *Ocimum* species viz. *Ocimum sanctum* L, *Ocimum basillicum* L. and *Ocimum gratissimum* L. were collected from the herbal garden of RMRC (ICMR), Belgaum, Karnataka and taken in the study.

**DNA extraction:**
Fresh leaves were placed on clean glass slide and their midribs removed using surgical blade. In case of seeds, the seed coats were removed with the help of forceps. 0.1g each of leaves and seeds from the same plant were taken for whole genomic DNA isolation in parallel.
The tissues were finely ground in mortar and pestle using liquid nitrogen. CTAB/NaCl method modified from [19] was followed for extraction of total genomic DNA from each sample. The pellets so obtained were re-suspended in 50 µl TE buffer and stored at 20°C until further use.

**DNA estimation:**
The quality and quantity of extracted DNA was determined using Nanodrop 1000 spectrophotometer (JH BioSciences) at 260/280 nm as well as visually by

carrying out horizontal gel electrophoresis with 2 µl of each sample loaded on 1% agarose gels stained with GelRed (Biotium). The agarose gels were visualized and documented under UV light using a gel documentation system with Alpha Imager software (Alpha Innotech Corporation, USA). The results, in terms of yield of DNA were analyzed by One way ANOVA followed by Dunetts *post hoc* test. $P \leq 0.05$ was considered as significant.

**Random Amplified Polymorphic DNA (RAPD) assay:**

RAPD fingerprinting assay was carried out with 30 ng of genomic DNA isolated from the leaves and seeds of each of the three species of *Ocimum* in a PCR thermal cycler (iCycler, BioRad Inc., USA) in 25 µl reaction volume with Plant RAPD primer RPi 1 (5'AAAGCTGCGG 3') (RPi C series, Bangalore Genei, India). The PCR mixture consisted of 30 ng of the extracted DNA added to 24 µl of PCR amplification mix consisting of 0.3µM primer, 200 µM of each dNTP (New England Biolabs, USA), 1.5 units of Taq DNA polymerase (New England Biolabs, USA) in 10X PCR buffer supplied (Tris HCl, pH 9.0; 15 mM $MgCl_2$). Amplification conditions were as follows: initial denaturation at 94°C for 3 min followed by 35 cycles of denaturation at 94°C for 54 sec, annealing at 43°C for 45 sec, and extension at 72°C for 2 min. with final extension at 72°C for 5 min.

The entire PCR amplified product was loaded on 1% agarose gels with GelRed (Biotium) dye for documentation. Each assay was repeated at least three times.

**Inter Simple-Sequence Repeat (ISSR) fingerprinting assay:**

ISSR fingerprinting assay was carried out with 30 ng of DNA isolated from the leaves and seeds of each of the three species of *Ocimum* in a PCR thermal cycler (iCycler, BioRad Inc., USA) in 25 µl reaction volumes with UBC primer 841 (5' GAG AGA GAG AGA GAG AYC 3'). The PCR mixture consisted of 30 ng (in 1µl) of the extracted DNA added to 24 µl of PCR amplification mix consisting of 0.3µM primer, 250 µM of each dNTP (New England Biolabs, USA), 400 µM of spermidine (Sigma, India), 0.48 units of Taq DNA polymerase (New England Biolabs, USA) in 10X PCR buffer supplied (Tris HCl, pH 9.0; 15 mM $MgCl_2$). Amplification conditions were as follows: initial denaturation at 94°C for 5 min followed by 45 cycles of denaturation at 94°C for 30 sec, annealing at 50°C for 45 sec and extension at 72°C for 2 min. with final extension at 72°C for 5 min.

The entire PCR amplified product was loaded on 1% agarose gels with GelRed (Biotium) dye for documentation. Each assay was repeated at least three times.

## RESULTS

**DNA quality:**

Sharpness and intensity of the DNA bands obtained from both the leaves and seeds of all the three species of *Ocimum* in agarose gel electrophoresis indicated that they are of good quality (Fig.1). The intensity of bands obtained from the respective seeds of each species was higher than those obtained from their leaf counterparts. Comparison of OD at 260/280 nm in spectrophotometer also showed readings between 1.0 to 1.5 for all samples indicating that the DNA were of good quality.

**DNA yield:**

The yield of DNA from seeds for each of the three species, and for each of the 5 times the experiment was repeated, was not only significantly higher ($P \leq 0.05$) but almost double than from the leaves. The quantity of DNA obtained from equal amounts of leaves and seeds from each of the three species of *Ocimum* are graphically represented in Fig. 2. Clearly, the seeds yielded more DNA than the leaves of the same weight for all the three species tested. The average yield of DNA from 100 mg of leaves was 190 ng/µl while 504 ng/µl of DNA was the average yield from seeds of the same weight.

**RAPD and ISSR fingerprinting assays:**

The DNA extracted from both seeds and fresh leaves was successfully used in amplification by PCR with both RAPD (not shown) and ISSR fingerprinting techniques (Fig. 3) indicating that the DNA obtained from either sample can be used for these applications. No differences were observed in banding patterns between DNA isolated from leaves and seeds of the same species. Differences however existed between the species of *Ocimum*, as expected.

## DISCUSSION

Although fresh leaves are considered as the best samples for extraction of DNA for subsequent downstream applications, out study showed that seeds can be better alternative. Being dry, it has longer shelf life and can be used after long periods for extraction of DNA. It does not generally require storage in freezers. Transportation of seeds is easier as the requirement of cold chain is not applicable in the case of seeds unlike that in case of fresh leaves. Most importantly, the yield of DNA in our study has been found to be higher from the seeds than from fresh leaves, and therefore smaller quantities of seeds will be required for obtaining required amounts of DNA. Interference in DNA extraction by large amounts of chlorophyll often results in reduced and inferior yield of DNA from fresh leaves [20] that can be overcome using seeds as the starting material. Although it is obvious that seeds of some plants may not be available at all times, may be difficult to process if too hard and bulky, and the yield

and quality may not follow the same pattern as has been found in this study with only a few species of *Ocimum*, this communication should help plant scientists to consider using seeds as alternative to fresh leaves for DNA extraction.

**ACKNOWLEDGEMENTS**
The authors are thankful to Ms. Asmita Gaonkar, B.Tech, Research Trainee, RMRC, Belgaum, for her laboratory assistance in the study. The study was carried out with the internal funds of the ICMR.

**REFERENCES**

[1] Arif I.A., M.A. Bakir, H.A. Khan, A.H. Al Farhan, A.A. Al Homaidan, A.H. Bahkali, M. Al Sadoon, M. Shobrak (2010) Application of RAPD for molecular characterization of plant species of medicinal value from an arid environment. Genet Mol Res, 9 (4): 2191-98

[2] El-Domyati F.M., A.A.Y. Rania, S. Edris, A. Mansour, J. Sabir, A. Bahieldin (2011) Molecular markers associated with genetic diversity of some medicinal plants in Sinai. J Med Plants Res, 5 (10): 1918-29

[3] Harisaranraj R., R. Prasitha, S.S. Babu, K. Suresh (2008) Analysis of inter-species relationships of *Ocimum* species using RAPD markers. Ethnobotanical Leaflets. 12: 609-613

[4] Singh A.P., S. Dwivedi, S. Bharti, A. Srivastava, V. Singh, S.P.S. Khanuja (2004) Phylogenetic relationships as in *Ocimum* revealed by RAPD markers. Euphytica. 136**:** 11–20

[5] Cimino M.T. (2010) Successful isolation and PCR amplification of DNA from National Institute of Standards and Technology (NIST) herbal dietary supplement standard reference material powders and extracts. Planta Med, 76 (5): 495-497

[6] Howard C., P.D. Bremner, M.R. Fowler, B. Isodo, N.W. Scott, A. Slater (2009) Molecular identification of *Hypericum perforatum* by PCR amplification of the ITS and 5.8S rDNA region. Planta Med, 75 (8): 864-869

[7] Kress W.J., K.J. Wurdack, E.A. Zimmer, L.A. Weigt, D.H. Janzen (2005) Use of DNA barcodes to identify flowering plants. PNAS, 102 (23): 8369-74

[8] Wang W., Y. Wu, Y. Yan, M. Ermakova, R. Kerstetter, J. Messing (2010) DNA barcoding of the Lemnaceae, a family of aquatic monocots. BMC Plant Biol, 10: 205

[9] Khalil R.M.A., K.A. Soliman, N.A.K.F. Rashed, S.A. Ibrahim (2010) Genetic polymorphism of some medicinal plants belonging to *Brassicaceae* using molecular markers. Egypt J Genet Cytol, 39: 41-55

[10] Sarwat M., S. Das, P.S. Srivastava (2008) Analysis of genetic diversity through AFLP, SAMPL, ISSR and RAPD markers in *Tribulus terrestris*, a medicinal herb. Plant Cell Rep, 27: 519–528

[11] Vijayanand V., N. Senthil, S. Vellaikumar, M. Paramathma (2009) Genetic diversity of Indian *Jatropha* species as revealed by morphological and ISSR markers. J Crop Sci Biotech, 12 (3): 115-120

[12] Atal, C.K., B.M. Kapoor (1989) Cultivation and utilization of medicinal plants. PID CSIR

[13] Chopra R.N., I.C. Chopra, K.L. Handa, L.D. Kapoor (1993) Indigenous drugs of India. UN Dhar Pvt. Ltd., Calcutta

[14] Gupta S.K., J. Prakash, S. Srivastava (2002) Validation of claim of Tulsi, *Ocimum sanctum* Linn. as a medicinal plant. Indian J Exp Biol, 40(7): 765–773

[15] Nadkarani A.K., K.M. Nadkarani (1976) Indian materia medica. Popular Prakashan Pvt. Ltd., Bombay

[16] Nagarajun S., H.C. Jain, G.S. Aulakh, (1989) Indigenous plants used in fertility control. In: Atal, C.K., Kapoor, B.M., eds. Cultivation and utilization of medicinal plants. PID CSIR, pp. 558

[17] Sirkar N.N. (1989) Pharmacological basis of Ayurvedic therapeutics. In: Atal, C.K., Kapoor, B.M., eds. Cultivation and utilization of medicinal plants. PID CSIR

[18] Warrier P.K. (1995) Indian Medicinal Plants. Orient Longman

[19] Keb-Llanes M., G. Gonzalez, B. Chi-Manzanero, D. Infante (2002) A rapid and simple method for small-scale DNA extraction in *Agavaceae* and other tropical plants. Plant Mol Biol Rep, 20**:** 299a–299e

[20] Reddy J. (2009) A comprehensive method to isolate high quality DNA from the cultivars of *Hibiscus.* Int J Biotechnol Appl, 1 (2):1-9

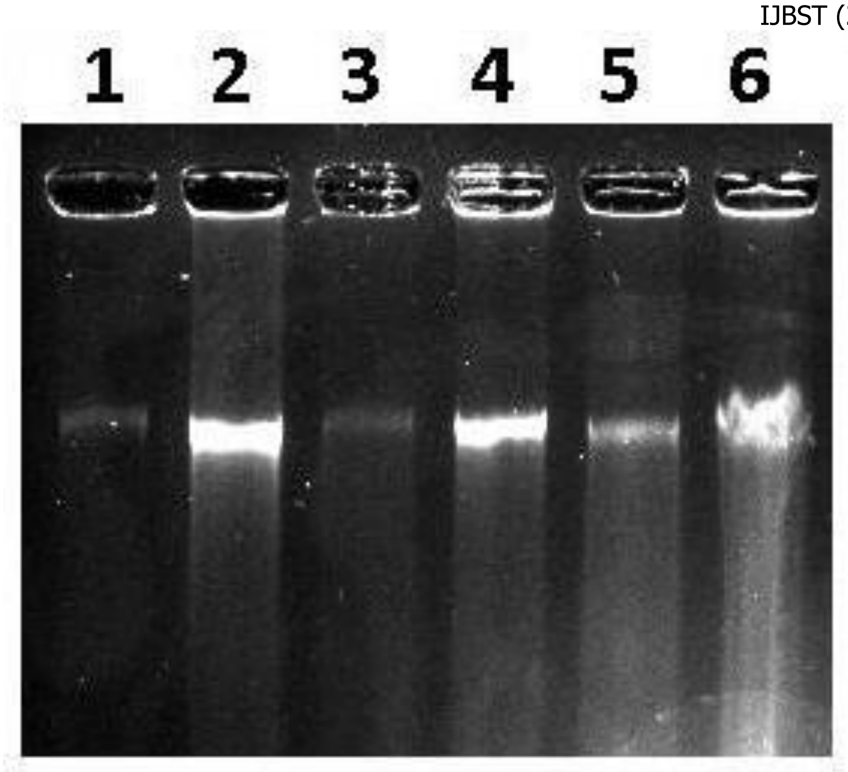


**Fig**. **1** Agarose gel electrophoresis of genomic DNA isolated from leaves and seeds of *Ocimum* species. Lane 1: *O.sanctum* (Leaf); Lane 2: *O.sanctum* (Seed); Lane 3: *O.gratissimum* (Leaf); Lane 4: *O.gratissimum* (Seed); Lane 5: *O.basilicum* (Leaf); Lane 6: *O.basilicum* (Seed)

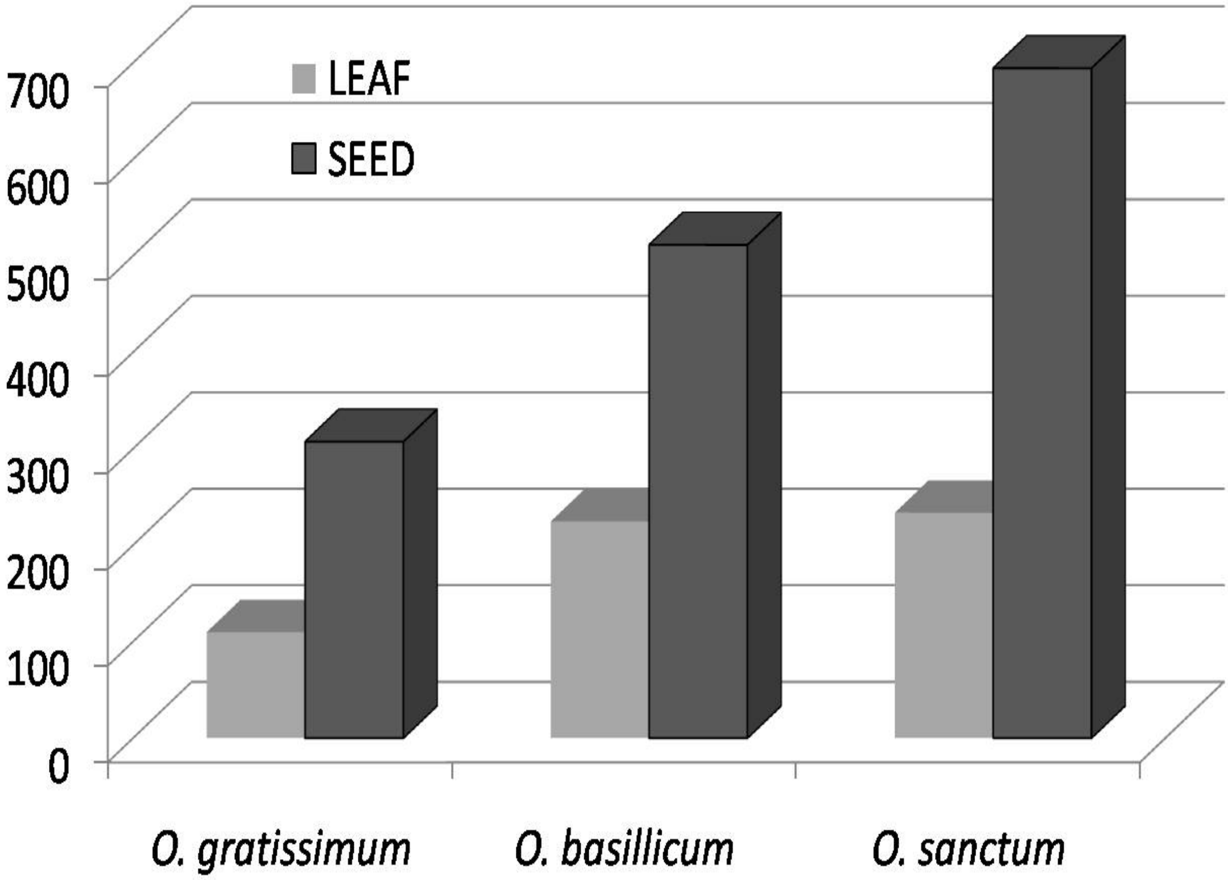


**Fig**. **2** Comparisons of DNA yield (concentrations in ng/µl from 0.1 g of sample) from *O. gratissimum, O. basilicum* and *O. sanctum*

ISSN: 0974 – 3987
IJBST (2011), 4(11):77-81

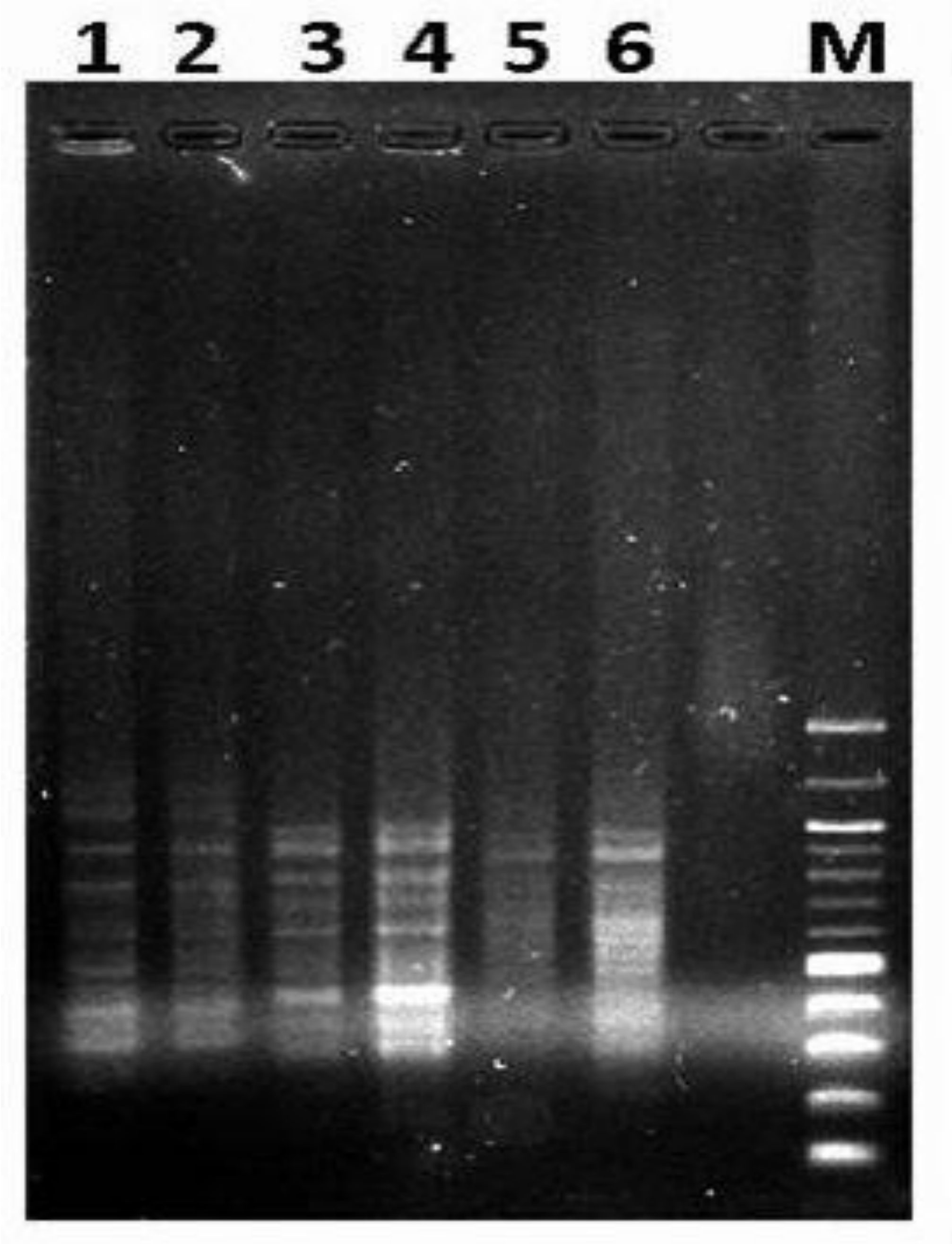


**Fig. 3** ISSR-PCR profiles of DNA obtained from leaves and seeds of *Ocimum* species. Lane 1: *O. sanctum* Leaf; Lane 2: *O. sanctum* Seed; Lane 3: *O. gratissimum* Leaf; Lane 4: *O. gratissimum* Seed; Lane 5: *O. basilicum* Leaf; Lane 6: *O.basilicum* Seed and Lane M: 100 bp molecular weight marker